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Differences in Relative Abundance of GnRH-I and GnRH-II in Granulosa Cells of Bovine Antral Follicles at Specific Stages of Follicular Development

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Objective

To characterize relative abundance of GnRH-I and GnRH-II mRNA within granulosa cells of bovine follicles at specific stages of follicular development.

Study Description

Beef cows were synchronized, and ovaries were collected at specific stages of follicular development [pre-selection (PRE), post-selection (POST), and post-selection 24 hours after luteal regression (POST-PG)]. All surface follicles were classified as small (<5mm), medium (5-8mm), or large (>8mm) and aspirated to collect granulosa cells. Large follicles from each animal were kept separate and all other follicles were pooled by size within animal (n=27, 27, and 18 for small, medium, and large). Total cellular RNA was extracted, and RT-PCR was performed for GnRH-I, GnRH-II, and GAPDH.

Take home points

Follicles of a less advanced stage in their development have greater mRNA abundance of GnRH-I compared to follicles of more advanced stages. Less advanced follicles are known to produce less estradiol. This study provides additional support of the inverse relationship of estradiol and GnRH within bovine follicles. Therefore, GnRH within antral follicles may be a key regulator of the follicle's ability to produce estradiol.

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Keywords: GnRH, granulosa cells, mRNA abundance, ovary

Differences in Relative Abundance of GnRH-I and GnRH-II in Granulosa Cells of Bovine Antral Follicles at Specific Stages of Follicular Development

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Abstract

Increased estradiol is the primary signal to initiate standing estrus, and cows that exhibit estrus prior to fixed-time artificial insemination have greater pregnancy rates than cows that do not. Therefore, understanding what factors may be present at the ovary that may regulate estradiol production is critical. A previous study reported that bovine follicles with greater follicular fluid concentrations of estradiol had decreased expression of GnRH-I and GnRH-II in granulosa cells (GC). The objective of this study was to characterize relative abundance of GnRH-I and -II mRNA within GC of follicles at specific stages of development. Beef cows were synchronized, and ovaries were collected at specific stages of follicular development [pre-selection (PRE), post-selection (POST), and post-selection 24 h after luteal regression (POST-PG)]. All surface follicles were classified as small (< 5mm), medium (5 - 8mm), or large (> 8mm) and aspirated to collect GC. Large follicles from each animal were kept separate and all other follicles were pooled by size within animal (n = 27, 27, and 18 for small, medium, and large). Total cellular RNA was extracted, and RT-PCR was performed for GnRH-I, GnRH-II, and GAPDH. Data were analyzed using the MIXED procedure of SAS. Across all follicles, GnRH-I and GnRH-II expression were not influenced by stage ($P = 0.27$) but were influenced by size ($P < 0.01$). Smalls (4.55 ± 0.39 and 3.91 ± 0.44, respectively) had greater expression ($P \leq 0.01$) of GnRH compared to mediums (0.83 ± 0.39 and 1.41 ± 0.44, respectively) and larges (0.52 ± 0.47 and 2.12 ± 0.54, respectively). There was also a stage by size interaction ($P < 0.01$). POST ($P < 0.01$) and POST-PG ($P \leq 0.08$) smalls had or tended to have increased expression compared to PRE smalls, but PRE mediums had increased expression ($P < 0.03$) compared to POST-PG mediums. When only the largest follicle for each animal was evaluated, stage of development influenced expression of GnRH-I ($P = 0.03$) but not GnRH-II ($P = 0.91$). For GnRH-I, PRE tended ($P = 0.09$; 2.29 ± 0.55) to have increased expression compared to POST (0.92 ± 0.55) and did have greater expression compared to POST-PG ($P = 0.01$; 0.11 ± 0.55). Thus, GnRH within antral follicles may be a key regulator of the follicle's ability to produce estradiol.

Introduction

The growth dynamics of follicles on the ovary involve four processes: recruitment, selection, dominance, and ovulation or death (Fortune, 1994). As follicles progress through the different developmental stages they secrete increasing amounts of estradiol. Estradiol is the primary stimulus for an animal to exhibit standing estrus (Vailes et al., 1992). Furthermore, cows that exhibited estrus prior to Fixed-Time AI had up to 27% increases in pregnancy rates compared to those that did not (Richardson et al., 2016). Therefore, investigation of ways to enhance estradiol production and expression of estrus presents an imperative area of research for the cattle industry. One method to accomplish this would be to identify factors present at the level of the ovary that may regulate estradiol production. Gonadotropin releasing hormone (GnRH) has been reported to have both stimulatory and inhibitory actions on steroidogenesis in the ovary (Sharp, 1982). A previous study by our lab reported that bovine follicles with greater

follicular fluid concentrations of estradiol had decreased relative expression of GnRH-I and GnRH-II in granulosa cells (GC) (Rich, 2017). Relative abundance of GnRH-I and GnRH-II mRNA in bovine GC was increased in follicles with decreased estradiol concentrations. Thus, the decreased abundance of GnRH-I and GnRH-II in large follicles and those with elevated concentrations of estradiol may indicate that GnRH is capable of regulating estradiol production in bovine antral follicles (Rich, 2017). The objective of this study was to characterize relative abundance of GnRH-I and -II mRNA within GC of follicles at specific stages of development. We hypothesized that as follicles were of an advanced stage of development that relative abundance of GnRH within the ovary would decrease.

Experimental Procedures

Experimental Design. A group of mature beef cows having normal estrous cycles were synchronized into specific stages of follicular development. Cows were observed for estrus, then during the midluteal period a new follicular wave was induced with an injection of GnRH (100 μg). Transrectal ultrasonography was performed daily to determine ovulation and initiation of a new follicular wave.

Tissue Collection. Immediately following slaughter or ovariectomy (Youngquist et al., 1995), all visible surface follicles were classified as small (<5 mm), medium (5–8 mm), or large (>8 mm). Follicular fluid was aspirated from all follicles, and GC were separated from the follicular fluid by centrifugation, placed in RNase Free tubes (USA Scientific), and snap frozen in liquid nitrogen. All samples were stored at -80°C. Total cellular RNA was extracted from the GC and RT-PCR was performed to determine relative abundance of mRNA for GnRH-I, GnRH-II, and GAPDH.

Follicle Classifications. Follicles were classified by size and stage. Follicle size classifications included small (<5 mm, n = 27), medium (5-8 mm, n = 27), and large (>8 mm, n = 18). For follicular stages, ovaries were collected following initiation of a new follicular wave, pre-selection (PRE). Ovaries were collected following selection of a dominant follicle (POST). An injection of $PGF_{2\alpha}$ (25 mg) following selection of a dominant follicle was administered and ovaries were collected 48 hours later before any animal initiated standing estrus (POST-PG).

Statistical Analysis. The mixed procedure of SAS was used to analyze relative abundance of GnRH-I and GnRH-II mRNA in GC by both size (small, medium, and large follicles) and follicular stage (PRE, POST, and POST-PG follicles) and was corrected for by GAPDH.

Results and Discussion

All follicles. Across all follicles, GnRH-I and GnRH-II were not influenced by stage ($P = 0.27$) but were influenced by size ($P < 0.01$). For GnRH-I and GnRH-II smalls (4.55±0.39 and 3.91±0.44, respectively) had greater expression ($P \leq 0.01$) compared to mediums (0.83±0.39 and 1.41±0.44, respectively) and larges (0.52±0.47 and 2.12±0.54, respectively; Figures 1 and 2). These results agree with previous research that reported a decrease in GnRH mRNA abundance as follicle size got larger (Rich, 2017).

Follicle size and stage interaction. There was also a stage by size interaction ($P < 0.01$). POST ($P < 0.01$) and POST-PG ($P \leq 0.08$) smalls had or tended to have increased expression compared to PRE smalls, PRE mediums had increased expression ($P < 0.03$) compared to POST-PG mediums.

This may be explained by the natural hierarchy of follicular development, where the dominant follicle(s) that would be present at the POST and POST-PG stages would be inhibiting the growth of the smaller follicles present at that time, and this could be mediated through GnRH.

Synchronized follicle. When only the largest follicle for each animal was evaluated, stage of development influenced expression of GnRH-I ($P = 0.03$) but not GnRH-II ($P = 0.91$). For GnRH-I, PRE tended ($P = 0.09$; 2.29±0.55) to have increased expression compared to POST (0.92±0.55) and did have greater expression compared to POST-PG ($P = 0.01$; 0.11±0.55; Figures 3 and 4). This provides evidence that not only do size and estradiol concentrations of follicles influence GnRH mRNA abundance (Rich, 2017), but this relationship is also seen at specific stages of development.

Implications

The relationship of estradiol and GnRH within antral follicles may regulate the follicle's ability to produce estradiol, and may play a role in the progression of follicles through the developmental stages of their growth.

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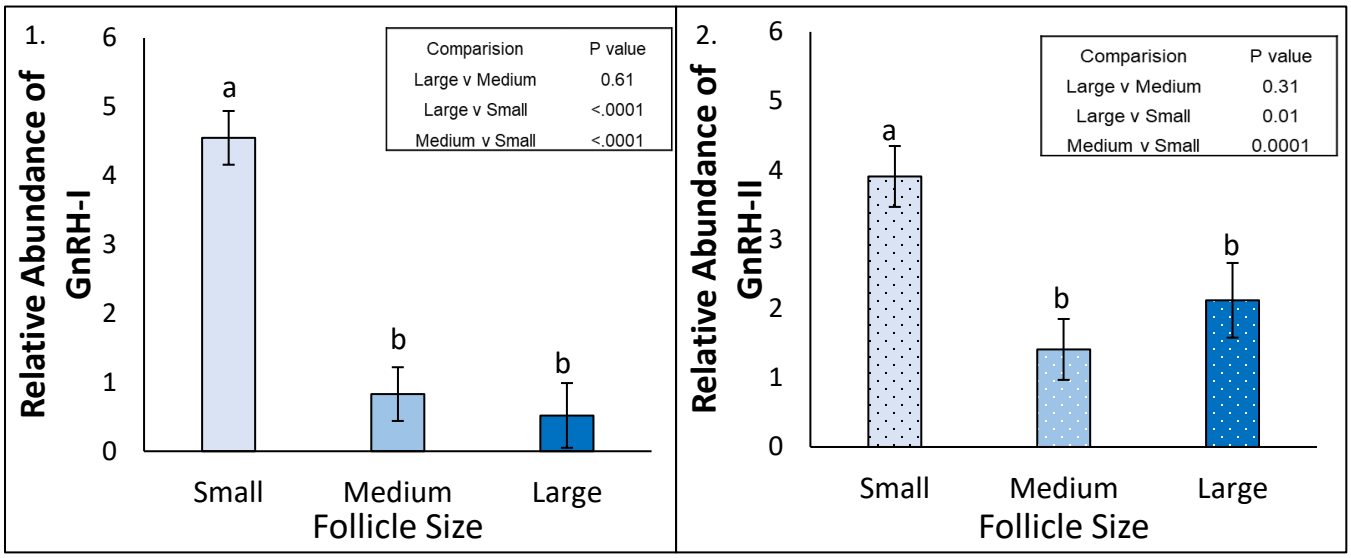
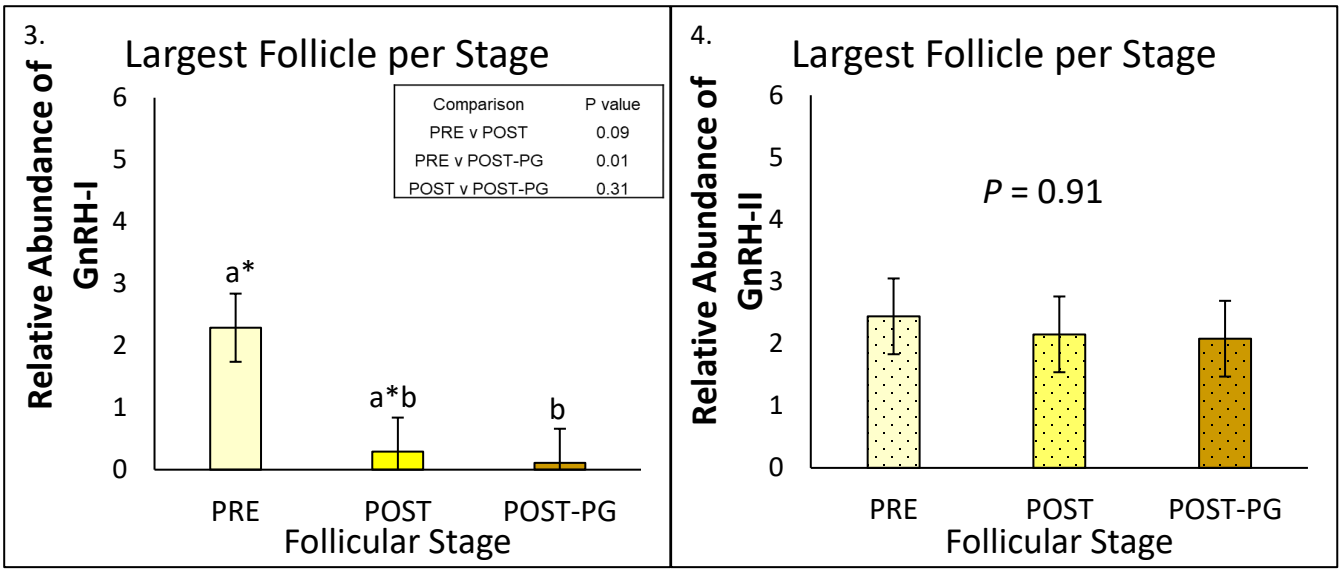


Figures 1 and 2. Relative abundance of GnRH-I and GnRH-II mRNA in granulosa cells of small, medium, and large follicles ($P < 0.01$).



Figures 3 and 4. Relative abundance of GnRH-I mRNA in granulosa cells of the largest/synchronized follicle per stage ($P < 0.03$; Figure 3). Relative abundance of GnRH-II mRNA in granulosa cells of the largest/synchronized follicle per stage ($P = 0.91$; Figure 4).

Acknowledgements

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